Room for all? - particulate surface area and bacterial activity in RAS

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Published in:
3rd NordicRAS Workshop on Recirculating Aquaculture Systems Molde, Norway, 30 September - 1 October 2015: Book of Abstracts

Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

3rd NordicRAS Workshop on Recirculating Aquaculture Systems
Molde, Norway, 30 September - 1 October 2015

Book of Abstracts

DTU Aqua report no. 301-2015

By Anne Johanne T. Dalsgaard (ed.)

DTU Aqua
National Institute of Aquatic Resources

The workshop is organized by the Nordic Network on Recirculating Aquaculture Systems (NordicRAS) in cooperation with the Technical University of Denmark (DTU Aqua) and Nofima

The workshop is supported by:
Nordic Council of Ministers
The Research Council of Norway
VRI Møre og Romsdal

Main sponsor:
BioMar A/S

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Grundfos DK A/S
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The granted support is hereby acknowledged

Title:	3rd NordicRAS Workshop on Recirculating Aquaculture Systems Molde, Norway, 30 September - 1 October 2015 Book of Abstracts
Author:	Edited by Anne Johanne T. Dalsgaard
DTU Aqua report no.:	301-2015
Year:	September 2015
Reference:	Reference: Dalsgaard, A. J. T. (ed.), 2015. 3rd NordicRAS Workshop on Recirculating Aquaculture Systems. Molde, Norway, 30 September - 1 October 2015. Book of Abstracts. DTU Aqua Report No. 301-15. National Institute of Aquatic Resources, Technical University of Denmark, 56 pp.
Cover photos:	Peter Lauesen and Martin Dam Kristensen.
Published by:	Technical University of Denmark, National Institute of Aquatic Resources Jægersborg Allé 1, 2920 Charlottenlund, Denmark Ph. + 45 35 88 33 00 E-mail: aqua@aqua.dtu.dk Web: www.aqua.dtu.dk
Download:	www.aqua.dtu.dk/publikationer
ISSN:	1395-8216
ISBN:	978-87-7481-215-9 (print) 978-87-7481-214-2 (web)

NordicRAS

3rd NordicRAS Workshop on Recirculating Aquaculture Systems

Book of Abstracts

Molde, Norway 30 September - 1 October 2015

DTU Aqua report no. 301-2015

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Preface

Welcome to the 3rd workshop on recirculating aquaculture systems by the NordicRAS Network (NordicRAS). This time the workshop is held in scenic Molde at the western coast of Norway. It is organized by NordicRAS in collaboration with the Technical University of Denmark (DTU Aqua) supported by Nofima as local organizer.

The aim of this biennial workshop is to bring together engineers, scientists, farmers, managers, equipment and systems manufacturers, consultants and other professionals with an interest in RAS to share ideas, present new findings, promote collaboration across sectors, and further promote the industry.

Recirculation components, technologies, system operation and farming practices have been optimized for years, and increasingly larger commercial systems are being built in the Nordic countries especially for production of salmonids, but also sturgeon and pike perch systems are seeing the light of day. Recirculating systems are, however, still complex to operate and new challenges arise as the intensity of recirculation increases and as new species are introduced. One of these challenges are particles that may accumulate in the systems, and the first day of the workshop will focus on how particles are generated, how they are measured, how they are removed, and how they interact with bacteria. This will be succeeded by as session on microbial water quality given the growing realization that it is possible to affect microbial dynamics in RAS, and that this may be of paramount importance for the well-being of the fish.

As the intensity of recirculation increases in the systems so does the concentrations of particles, nutrients and other components that are not fully removed by mechanical, biological, ozone, UV or other types of in-line treatment devises but solely balanced via make-up water. The effects of this on water quality and on fish performance and welfare are touched upon on the second day of the workshop. The workshop will finalize with a session on integrated system approaches. The prospective of establishing complete systems that can also handle intake -and especially discharge water and sludge is gaining more and more attention as system size increases while discharge legislation, at best, remains the same.

The program will be tight as “always”, including 36 interesting presentations in the 1½ days the workshop lasts. In addition to the presentations, there will be a few posters introducing new research systems and potential, cross-country collaboration opportunities. The posters will be located in the exhibition area at the Scandic Seilet hotel lobby where also some companies have the opportunity to present themselves to the workshop audience.

BioMar is again main commercial sponsor of the workshop for which we are very grateful, as we similarly are for the commercial sponsorships from Grundfos and SalmoBreed. In addition to this, we thank Norden, the Norwegian Research Council, and VRI Møre og Romsdal for their financial support without which the workshop would never have taken place.

At the time of writing, there were 212 registered participants from 28 countries around the world! This is very overwhelming and way beyond what we had ever dreamt of when starting NordicRAS, and we hope and believe that your days in Molde will be worthwhile. Have a great workshop.

On behalf of NordicRAS,
Anne Johanne T. Dalsgaard, DTU Aqua

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Presented at the

3rd NordicRAS Workshop on

Recirculating Aquaculture Systems

Molde, Norway

30 September - 1 October 2015

Recirculation feed for Atlantic salmon

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ABSTRACT

The RAS production of salmon smolts in both Norway and Chile has been steeply increasing for more than a decade. This has been caused partly by an increase in number of salmon smolts produced, but also by a trend of growing smolts bigger (up to 400 grams or more) prior to sea cage stocking. As a consequence there is a demand for developing bespoke salmon smolt feeds for RAS with focus on:

• Superior growth performance (SGR)

• Improved feed conversion ratio (FCR)

• High nutrient digestibility and retention

• Stabilizing faecal matter

-in order to safely maintain high stocking densities and fast production cycles and reduce discharge of both organic and nitrogenous waste to a minimum without jeopardizing overall feed performance. Stabilization of fecal matter will not be treated in this presentation.

From a number of preceding trials, one experimental RAS diet was chosen to be trialled against two commercially available diets for Atlantic salmon smolt (*Salmo salar*) in a growth trial using triplicate $1m^3$ tanks. Both commercial diets were known to have been previously used for salmon smolt culture in RAS. The three diets were fed to salmon smolt growing from 20 – 175 grams over two time periods with a combined duration of 118 days. Fish were fed ad libitum for 24 hours per day at a temperature of 14°C. Light regime was 24L:0D throughout the trial.

Fish fed the experimental RAS diet showed significantly lower FCR values (0.75) compared to the commercial diets (both 0.78), while no significant differences were found in SGRs between dietary treatments (2.06 – 2.11% d^{-1}). Protein and lipid digestibilities of the two commercial diets (88.7-90.3% and 95.9-97.2%, respectively) were significantly lower than observed in the experimental RAS diet (91.7% and 98.0%, respectively). Similarly, fish fed the experimental RAS diet displayed an improved retention of digested protein (55.2%) compared to fish fed the two commercial diets (51.9 – 52.5%).

Collectively, these dietary measures allowed a reduction of nitrogen excreted via faeces and gills/urine of 20.5-33.6% and 10.6-13.1% per kg produced fish, respectively, and a reduction of dry matter feces excretion between 1.9-6.1% when using the proposed recirculation diet.

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When the shit hits the fan: diet composition, indigestible binders and fecal stability

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ABSTRACT

The dietary requirements of Atlantic salmon are well known, and feeds may incorporate a variety of raw materials to achieve optimal nutrient composition. Considerations of availability and price have driven a trend in which marine ingredients are increasingly substituted with raw material of vegetable origin. The introduction of RAS to salmon smolt production presents new challenges for feed technology, as these systems have limited capacity for handling organic waste. Initial mechanical treatment of waste is relatively uncomplicated, with low investment and running costs. The following biological treatment, by comparison, is costly, labour-intensive and much more challenging when it comes to controlling key water quality parameters. The efficiency of biological treatment depends on organic load, and thus improved mechanical filtration that reduces the amount of organic matter in the water is highly desirable. Feed designed for RAS should meet the nutritional requirements of the fish but also support particle removal by maximizing the mechanical stability of fecal waste.

A range of experimental salmon feeds with varying compositions were developed using raw ingredients including soya protein concentrate (SPC), corn concentrate, pea protein concentrate, wheat gluten and two types of indigestible binders in varying quantities. Twenty one diets were screened in salmon parr growth trials. Feed intake was measured at tank level, and at the end of the trial values for growth rate, feed conversion, digestibility and nutrient retention were calculated and intestinal histology of the fish was examined. At the final sampling, rheological properties of fecal material were measured for all treatment groups.

The results from the trials give a wide range of parameters on which the diets can be evaluated, and no single diet excelled on all counts. The different combinations of raw materials indicate some positive effects of SPC on rheological properties of the feces at high inclusion rates, but this outcome has to be treated carefully because of the potential detrimental emulsifying properties of SPC. The most consistent improvements were achieved by one of the binders tested, suggesting that recipes using a variety of raw ingredients easily can be adapted to RAS requirements simply by adding a binder. However, the possibility that mechanically stabilization of chyme may damage fish intestinal tissues must also be considered. Our results revealed that binder inclusion did affect the mucus layers in the mid gut of salmon smolts, but only one trial diet, a commercially available control supplemented with guar gum, yielded any significant negative effect on fish intestines.

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First experiences of floating faeces and its rapid removal in RAS

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ABSTRACT

Previous extensive laboratory experiments and a commercial-scale trial in a semi-recirculating aquaculture system (RAS) have tested a novel approach to controlling fish waste, using cork as a functional feed additive for the production of floating faecal casts. The results suggest the approach has great potential, not least in terms of efficiency.

The present study transferred the trial to a low water exchange RAS. A commercial diet producing feces in the upper recorded density range (1,034 ± 0,0042 g/cm^3) was used as a control. The addition of 2.5% cork to the control diet resulted in stable and consistently floating faecal casts with a mean density of 0.993 ± 0.003 g/cm^3.

The experiment was carried out in duplicate RAS, each stocked with 500 rainbow trout (*Oncorhynchus mykiss*). Fish were fed six days a week until apparent satiation over a period of 120 days. Stocking density increased from 10 kg/m^3 at the beginning of the experiment to a final density of 75 kg/m^3. Mean final fish weight was 567 g and did not differ statistically between systems.

Solid waste from the control system was collected in a pit before being transported to a drum filter. The tanks of the system in which the experimental cork diet was fed were additionally equipped with simple outlet pipes at the water surface, which transported the floating feces directly to the drum filter.

The effectiveness of solid removal for floating and normal (control) feces and the consequences for water quality parameters and waste treatment performance in the system were examined. Mechanical removal efficiency of cork-treated wastes was more than four times greater than that achieved in the control (89% vs. 20%). Total ammonia nitrogen (TAN) and nitrite nitrogen levels were comparable during the first weeks of the experiment. However, when the biofilters reached their capacity, TAN and nitrite removal was more efficient for the cork-treated system than the control. Physiological assays indicated no pathologic tissue alterations associated with the experimental diet and growth, survival and feed conversion were unaffected.

In conclusion, by simultaneously addressing environmental, welfare and economic concerns, a minimal feed-mediated density modification of fish faeces improves nearly all aspects of water quality investigated, with important implications for the future of modern closed fish farming.

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Feed composition affects sludge as a resource for denitrification

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ABSTRACT

Organic waste produced by fish (i.e., sludge) can be used as a carbon source (C) for denitrification, reducing the potential need for external carbon sources in on-farm nitrogen removal. By using sludge for denitrification, the discharge of organic matter and nitrate may be reduced, there may be no need to buy external carbon sources, energy for transport of waste is avoided and moreover, the waste is treated at the end of the process chain rather than being displaced to another environment (end-of-pipe concept). The capacity for denitrification depends on the presence of readily available carbon sources e.g., in the form of volatile fatty acids (VFAs) and alcohols, and on the C:N ratio. Volatile fatty acids and alcohols can be generated from sludge via hydrolysis and fermentation.

Undigested feed is by far the main source of organic waste in RAS, and knowing the digestibility of the feed makes it possible to predict the nutrient composition and consequently the organic matter content of the produced sludge. The purpose of the current study was to examine the effects of fish feed composition on the types and dynamics of readily available carbon sources obtained from concomitant hydrolysis/fermentation of sludge from rainbow trout fed diets with different protein:energy (P:E) content.

Rainbow trout faeces from fish fed 5 diets with different dietary P:E ratios were used to evaluate the net production (quantity and quality) of VFAs and ethanol. The faeces were hydrolyzed and fermented for 7 days in 1L batch reactors under anaerobic conditions. Samples for VFA and ethanol analysis were obtained daily and measured by HPLC and test kits. Values were converted to COD units, corresponding to the amount of readily available carbon sources.

Four to five days of hydrolysis/fermentation was required to achieve the maximum yield of VFAs and ethanol. The composition and quantities of readily available carbon sources changed according to diet composition and duration of the hydrolysis/fermentation process. The total yields obtained did not show statistical differences between dietary treatments, but diets with low P:E ratios produced more butyric acid and ethanol whereas diets with high P:E ratios produced more acetic and valeric acid.

Different P:E ratios in the diets affected the types of readily available carbon sources produced and the conditions of incomplete anaerobic digestion (e.g. pH). The results verified that it is possible to influence and optimize the quantity and quality of readily available carbon sources produced from sludge by manipulating the composition of fish feed, enabling an accumulation of intermediate organic acids (propionic, butyric and valeric acids) as well as more reduced end products (acetic acid and ethanol). Based on the obtained results an industrial scale side stream hydrolysis/fermentation reactor was built for evaluating the capacity of performing single-sludge denitrification at a commercial farm.

Acknowledgements: This research was funded by The Ministry of Food, Agriculture and Fisheries of Denmark and by the European Union through The European Fisheries Fund (EFF).

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Biofilter effects on micro particle dynamics

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ABSTRACT

Accumulation of fine particulate organic matter in recirculating aquaculture systems (RAS) is a balance between system input (from feed to waste), internal transformation, removal and dilution. The mechanisms leading to fine particle accumulation in RAS are not fully understood, and neither is the potential influence of biofilters in this respect.

This study describes the effect of fixed bed biofilters (FBB) and moving bed biofilters (MBB), on particle size distribution and organic matter. It was assessed in an 8.7 m^3 RAS with four equal biofilters (two FFB and two MBB) stocked with rainbow trout (*Oncorhynchus mykiss*), and operated under constant feed loading conditions (1 kg feed/m^3 of make-up water) for more than 3 months. Carrier media was similar in shape and specific surface area for both reactor types, differing only in specific density.

Particle concentration was reduced by ca. 200 particles/mL in FBBs, and increased by ca. 250 particles/mL in MBBs. In FBBs, a 10 % reduction in particle concentration also represented a 10 % reduction in total particle surface area and particle volume. In MBBs, a 10 % increase in particle concentration also represented a 10 % increase in total particle surface area, but had no effect on total particle volume. A volumetric reduction of particles > 100 µm, and an equivalent volumetric increase of particles < 40 µm showed that MBBs produced fine particles by disintegration of larger particles. A constant volumetric removal of particulate matter by FBB in all size classes demonstrates their function as secondary particle removal units.

Net removal of organic matter occurred at the same rates in both modes of operation. However, FBB removed a higher amount of dissolved BOD_5 than MBB, while MBB removed a higher amount of particulate BOD_5 than FBB. All filters performed with stable nitrification rates when operated together or separately, with net removal of ammonia and nitrite being larger in FBB than in MBB.

Differences in biofilm formation, development, and maintenance, coupled to reactor flow characteristics are discussed in relation to the fate of micro particles and organic matter when operating FBBs or MBBs.

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Evaluation of membrane treatment effect on water quality in recirculating aquaculture systems (RAS) for Atlantic salmon post-smolts (*Salmo salar*)

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ABSTRACT

Accumulation of solids is a challenge in recirculating aquaculture systems (RAS), and could affect the water quality, microbial community and fish health. Typical particle removal systems only manage to remove particles larger than 40-60µm leaving the fine solids. There is clearly a need for advanced particle removal system. By integrating a membrane unit removing particles <1µm, accumulation of microfines can be avoided. This study evaluated effects on water quality.

This study was done at Nofima Centre for Recirculation in Aquaculture. A membrane modified RAS (mRAS) was compared to a conventional RAS (cRAS) for production of Atlantic salmon post–smolts. A membrane (AQUAFLEX 64®, Pentair/X-flow) with nominal pore size of 20 nm and total area of 60 m^2 was connected to the outlet of the biofilter, and clean permeate water was returned back into the system. This setup filtered 5% of the total system flow for 10 h/d, corresponding to 50% of the total system volume and 2.5 m^3/h of permeate. The starting fish weight and tank density were 250 g and 50 kg/m^3, respectively.

Analysis of physiochemical water quality parameters, total suspended solids (TSS), particle size distribution (PSD), turbidity, denaturing gradient gel electrophoresis (DGGE) and total number of bacteria was conducted. To investigate the TAN-removal efficiency in the biofilter, and the potential correlation to the organic material in the water, nitrogen compounds and total organic carbon (TOC) were measured. The concentration of bacteria in the water was determined by flow-cytometry, and microbial community structure was investigated using a 16S rDNA amplicon/DGGE strategy.

The membrane improved the water clarity and had a significant effect on several water quality parameters. Turbidity, TSS and TOC were lower in mRAS compared to cRAS ($p=0.05$) (Fig. 1, A - C, respectively). Furthermore, the treatment had a positive effect on the nitrogen removal during the whole experiment (Fig. 1, D). Bacterial numbers were significantly lower in mRAS compared to cRAS. Further results on water quality and microbial community structure will be presented.

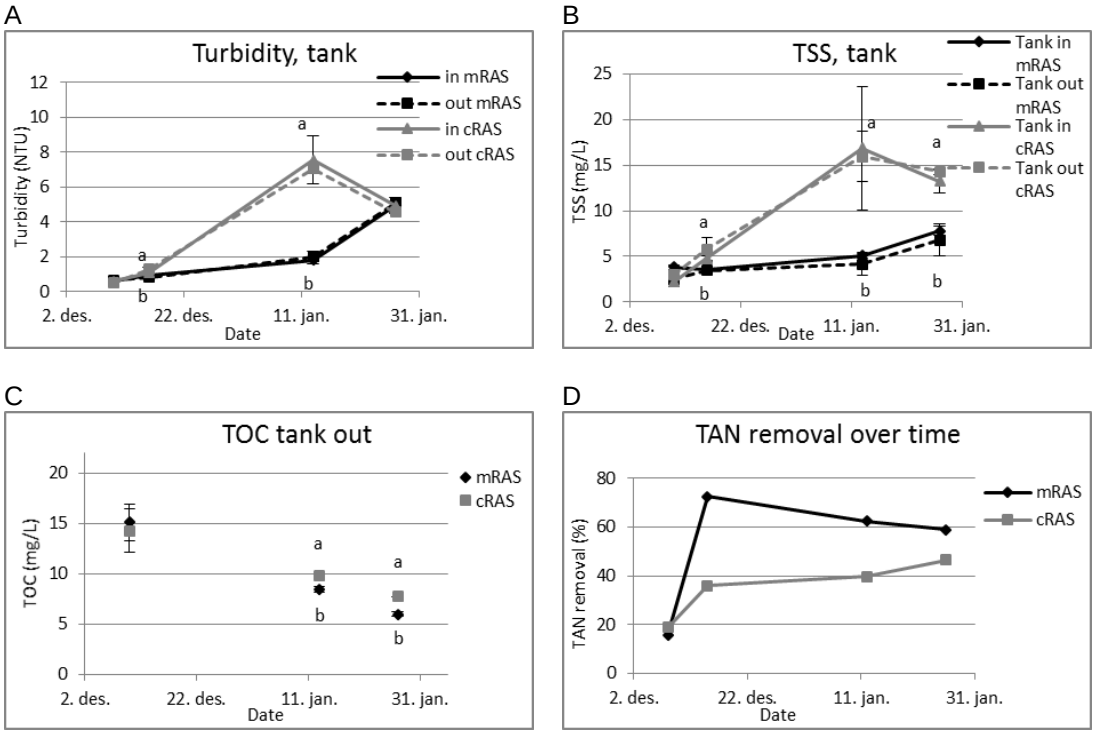

Figure 1. Turbidity tank (A), TSS tank (B), TOC tank (C) and TAN removal (D). Values represent average ± SD while different letters indicate significance.

Acknowledgements: This study was funded by a Research Council of Norway strategic institute program, pr. 194050/F40 “Development of future controlled-environment aquaculture”. The membrane was supplied by Pentair.

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Monitoring RAS organic matter by fluorescence EEM spectroscopy

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ABSTRACT

One of the challenges to recirculating aquaculture is the risk of operational failure, which may arise from poor water quality conditions. Organic matter (OM) can have a significant impact on microbial conditions and treatment process performance, and although current RAS monitoring methods sometimes include analysis of bulk organic matter, the organic matter within RAS is derived from a range of sources, each varying in relative importance with time. Bulk organic matter measurements alone may not provide an adequate estimate of how organic matter character may be fluctuating and potentially influencing RAS performance.

Individual components of fluorescent dissolved OM (FDOM) have been shown to correlate well with chemical and microbiological indicators that are important RAS parameters, though many are unable to be monitored online or analysed within a reactive timeframe. Fluorescence spectroscopy, however, is a fast, sensitive and non-destructive analysis technique, and hence shows great potential in its application to real-time monitoring of RAS. The aim of this study was to test if the technique can identify characteristic FDOM fractions from a complex RAS matrix, and to outline the potential of using fluorescence as a sensitive monitoring parameter of RAS water.

Four identical freshwater RASs, operating at four constant daily feed loads (125, 250, 375 or 500 g) were analysed by fluorescence EEM spectroscopy and DOC to study changes in OM at different feed levels. All EEM processing was carried out using the drEEM toolbox within Matlab software (MathWorks). The resulting dataset was then subjected to parallel factor (PARAFAC) analysis.

EEM-PARAFAC analysis was able to describe changing FDOM components in RAS: three components linearly correlated to feed input; one component correlated to feed input but showed accumulation with increased feed inputs; and one component correlated to tap water.

From a comparison of theoretical calculations and observed values, the fluorescence components were found to originate from three sources: 1) feed input; 2) influent tap water; and 3) organic matter produced by the fish and treatment processes. There was a considerable difference between the components as to the degree they originate from these sources.

Although DOC increased linearly with feed loading, the fluorescence components did not all adhere to the same linear relationship. This implied that fluorescence EEM spectroscopy may offer a more detailed approach to monitor the accumulation of bioavailable organic matter in RAS.

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Humic substances in recirculating aquaculture systems and their effect on fish health

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ABSTRACT

Most of today's recirculating aquaculture systems are semi-closed systems where, due to limited water use and high fish stocking densities, biosecurity is of prime concern. As compared to semi-closed systems, zero-discharge systems, operated by our group, include an additional anaerobic treatment step where, among other processes, organic matter is digested and nitrate is converted into nitrogen gas.

More than a decade of operation has revealed relatively few disease outbreaks in these systems. A unique characteristic of these zero-discharge systems is their dark brown water color as a result of the accumulation of humic substances – natural organic compounds which are end-products of organic matter degradation. Among the alleged benefits of these substances are their antiseptic and immunostimulating properties in organisms, including fish.

We characterized and quantified humic substances in a zero-discharge aquaculture facility stocked with hybrid tilapia (*Oreochromis niloticus* x *O. aureus*). By means of fluorescent analyses, four different types of humic substances could be identified. Concentrations of these substances in the culture water increased with time, mainly due to their production in the organic-rich digestion basin. In addition to the culture water, humic substances were also detected in blood withdrawn from fish cultured in the system.

An evaluation of the protective effect of humic substances was conducted by exposing Koi carps (*Cyprinus carpio*) to three sources of humic substances (added to the feed and to the culture water) and challenging them with atypical *Aeromonas salmonicida* - a major pathogen in Israeli aquaculture. All three sources of humic substances showed a considerable protection in Koi against this pathogen as compared to control fish.

Finally, we examined the direct effect of humic substances on the growth performance of two pathogenic bacteria, atypical *Aeromonas salmonicida* and *Streptococcus iniae,* as well as a crude bacterial culture originating from the system's culture water. Growth medium amended with humic substances from various sources resulted in growth inhibition of the pathogenic bacteria while growth of bacteria originating from the culture water was not affected.

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First findings on the effects of suspended solids in recirculating trout aquaculture on selected health parameters

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ABSTRACT

Particulate waste is generally removed from RAS by mechanical filtration, but the technique invariably allows some small, unscreenable particles to remain in the system, where they accumulate over time. High levels of fines are widely regarded as harmful to stock health and welfare. However, little is known about the direct impact of these accumulating particles on stress, performance or health parameters of fish. Furthermore, there are no defined threshold values at which sizes or concentrations fines start to become harmful in RAS. In order to improve RAS it is essential to learn more about the exact impact of accumulating particles on fish health and system efficiency.

In the present investigation, rainbow trout were housed in two replicate RAS. After an acclimatization period of three weeks, particle accumulation of one system was artificially increased by a multiple factor, while the parallel system remained under normal conditions as a control. A decisive and unique criterion for the investigation was the isolation of particle accumulation effects from other debilitating water parameters. In both systems, levels of ammonium, nitrite, pH, CO_2 etc. were continuously monitored and adjusted to levels deemed to have no impact on fish health or performance. The impact of accumulating particles on fish health was examined using a wide range of parameters. HSP70 and plasmacortisol concentration were ascertained by ELISA to detect changes in stress level. Cell count, differential blood count, hematocrit, leukocrit and hemoglobin content were analysed for hematological abnormalities. Fin damage was used as indicator of fish welfare. Particle accumulation was monitored in terms of TSS concentration and particle size distribution, and in a novel approach, the shape and structure of particles was investigated using a Particle Insight Size and Shape Analyzer and digital image analysis.

The results allow size distribution to be linked reliably with particle shape data and the potential impacts of accurately profiled waste on fish to be examined.

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Room for all? - particulate surface area and bacterial activity in RAS

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ABSTRACT

Suspended particles are considered to have a range of adverse effects in RAS. They consist largely of organic matter deriving from faces but biofilter flocks/slough off and uneaten feed also contribute. In particular, fine suspended solids and colloids contribute to the particulate community since they are not removed by traditional mechanical filtration technology and therefore tend to accumulate in the system. Bacteria generally attach to particles surfaces. Since particle numbers are high and given the high surface:volume ratio of fine solids, the heterotrophic bacterial carrying capacity in RAS is also high. It may therefore be anticipated that the bacterial load increases with increasing loading of suspended organic particles providing additional substrate and surface area. To examine the validity and extent of the assumed relationship between particulate surface area and bacterial activity, independent observations from different intensity RAS were compiled.

Grab samples from 4 independent RAS of different intensity were collected and analyzed for total and dissolved BOD_5, bacterial activity, particle counts and particle size distribution. Bacterial activity was derived from the hydrolase activity in the samples (Bactiquant®-Water, Mycometer A/S, Denmark), while the distribution of particles within the 5 - 400 µm size range was measured using an optical particle counter (AccuSizer™ 780 SIS, Particle Sizing Systems Inc., USA). The total particulate surface area (TSA; mm^2) was calculated based on the counts in each size-class, and particulate BOD_5 was calculated as the difference between total and dissolved BOD_5.

Bacterial hydrolase activity generally increased with increasing recirculation intensity, being roughly a factor 10 higher in the highest intensity RAS compared to the other systems. Relatively strong positive, linear correlations between organic matter and particulate surface area; between bacterial hydrolase activity and particulate BOD_5; and between bacterial hydrolase activity and particulate surface area was demonstrated for all RAS with feed loadings (recirculation intensities) between 23 and 1 m^3/kg feed, while there was no tight relationship at the highest recirculation intensity (0.32 m^3/kg feed) independently of microscreen configuration.

The results indicate that microbial activity/abundance in RAS depends on particulate surface area until a certain level is reached in intensive systems where bacterial activity is no longer directly dependent on particulate surface area.

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Beneficial fish-microbe interactions: the fourth dimension of RAS

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ABSTRACT

RAS has traditionally been used for three reasons: saving energy, saving water and handling of waste. By ecological reasoning and experimental data we argue that RAS has a huge potential as a microbial management method to secure beneficial fish-microbe interactions. Such interactions are a major reason for the low survival, growth and reproducibility in marine larviculture, but may also play a role in the significant mortality of smolt the first weeks after transfer to seawater.

Reasoning based on empirical data from hatcheries and ecological theory (the r/K-concept) made us hypothesis that traditional hatcheries set up selection for r-strategic opportunist due to frequent perturbations of the growth conditions of the bacteria. All detrimental bacteria are r-strategist, and thus the rearing technology is a main reason for the microbial problems we experience. To reduce the presence of opportunistic r-strategist the rearing technology and practice should be modified in a way which creates K-selection; i.e. low substrate availability per bacterium and strong competition for resources. K-selection can be obtained in two different ways; 1) In flow-through systems by controlled recolonization using a biofilter to secure competition for nutrient and thus K-selection before the water enter the fish tanks (Microbially Matured Systems; MMS). 2) In RAS the biomass of bacteria in biofilters will stabilize at a level were nutrient limitation and thus competition is strong, i.e. K-selection. However, K-selection will in both cases require appropriate architecture and operation conditions.

Experiments with fish support our hypothesis. Firstly, the two proposed K-selection system, MMS and RAS, give bacterial community composition of incoming water to the fish tanks that are significantly different from the bacterial communities in traditional flow-through systems (FTS). Moreover, the fraction of opportunistic bacteria is lower under K-selection conditions. A large number of experiments with MMS, some with RAS and one experiment comparing r-selected FTS with K-selected MMS and RAS, all indicate that K-selected microbial communities positively affects the performance of marine fish during larval rearing. Positive effects include increase in appetite, early onset of growth, higher growth rates, better survival and improved tolerance to stress.

Our r/K-selection hypothesis is supported by all experiments conducted so far, but a limited number of experiments have compared RAS and FTS. No experiments have been done with salmonids. For a proper verification of the r/K-selection hypothesis for RAS more experiments are needed, and they should include studies of the consequences system architecture.

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A novel real-time bacteria sensor for monitoring water in recirculating aquaculture systems

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ABSTRACT

Grundfos Holding has introduced an innovative approach to monitoring bacteria in aquaculture water. The fully automated, reagentless sensor delivers real-time results which can be accessed from common data platforms such as SCADA, web and mobile. The sensor automatically retrieves water samples every few minutes and works 24/7. The software includes real-time data acquisition, a patented 3D-scanning technique for advanced image analysis and automated data treatment. It has been integrated with a complex particle and bacteria image library for object recognition and counting. This combination of hardware and software technologies is able to differentiate between bacterial cells and non-bacterial particles with around 85% accuracy.

The bacteria sensor has been tested for a period of eight months at an inland pike perch farm (Egtved, Denmark), consisting of numerous recirculating aquaculture systems (RAS). The fish farm belongs to AquaPri, a Danish company that produces mainly trout, pike perch and eel. The investigated RAS included a fish tank, a drum filter, a biofilter, a trickling filter and a UV disinfection unit. Additionally, chemical disinfection with peracetic acid was performed periodically. The bacteria sensor monitored the water quality from the outlet of the fish tank, after passing the drum filter.

During the entire testing period, the bacteria sensor showed a stable operation and reflected daily variation of water quality resulting from both fish activity and system maintenance. Figure 1 illustrates an example three-week period of the performed experiment.

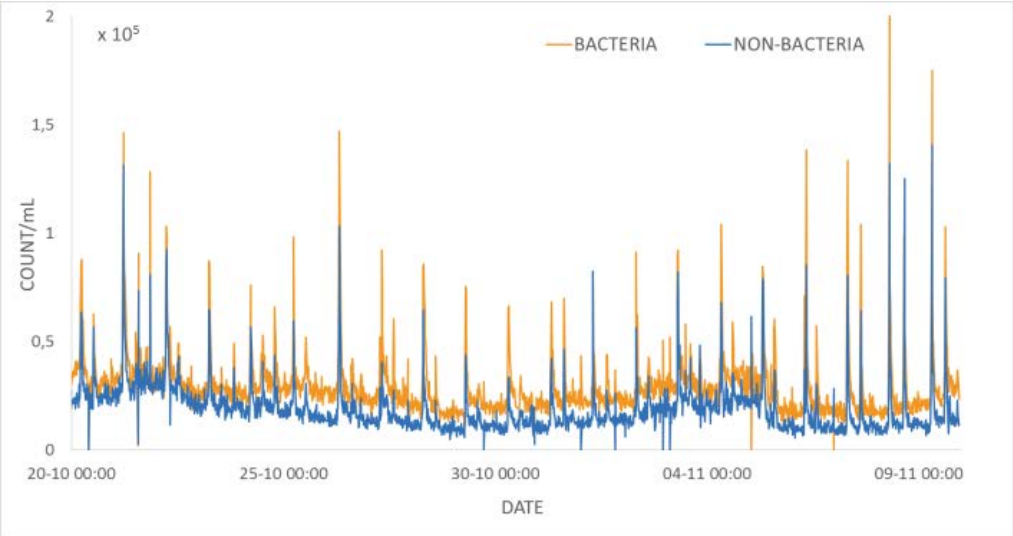

Figure 1. Bacteria and Non-bacteria counts in RAS water during a three-week period.

The baseline of bacteria count in the monitored water was in range of 2.0-4.0 E+04 cells/mL. The observed daily peaks were related to the daily routine like tank cleaning and fish feeding. The slightly elevated bacteria level observed from November 2nd to November 4th resulted from poor system maintenance performed during the weekend by less experienced staff. Chemical disinfection performed on November 5th initiated a new equilibrium state of the RAS, with a lower bacteria level.

The bacteria sensor has proven to detect even very slight changes in water quality during the tests in recirculated aquaculture systems. The outstanding sensitivity combined with real-time response makes the sensor a true innovation in monitoring RAS performance.

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Microbial community dynamics in three RAS with different salinities for production of Atlantic postsmolt

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ABSTRACT

Fishes share their living medium with high loads of bacteria. The microbial environments in aquaculture systems are fundamentally different from those of the fishes' natural environments, and how this affects fish health is not known. We previously demonstrated that stable microbial environments improve the growth and survival of reared marine larvae. It is therefore of interest to understand and ultimately to control the microbial community dynamics in the rearing system. However, the microbial ecology in a RAS is complex, and to obtain optimal microbial conditions, not only fish welfare, but also an efficient nitrification process in the bioreactor need to be considered. In this study we investigated microbial community dynamics in three RAS operated at different salinities.

Atlantic salmon postsmolts were produced in three RAS operated at distinct salinities (12, 22, and 32‰) in an experiment investigating effects of salinity and fish exercise on health, welfare, performance and water quality. Samples for microbial community analyses were taken from rearing water (in- and outlet from triplicate rearing tanks), moving bed bioreactor water (in- and outlet), and bioreactor biofilm carriers from each system at three time points spanning a 5 months period. Bacterial communities were analyzed using a 16S rDNA amplicon/DGGE strategy.

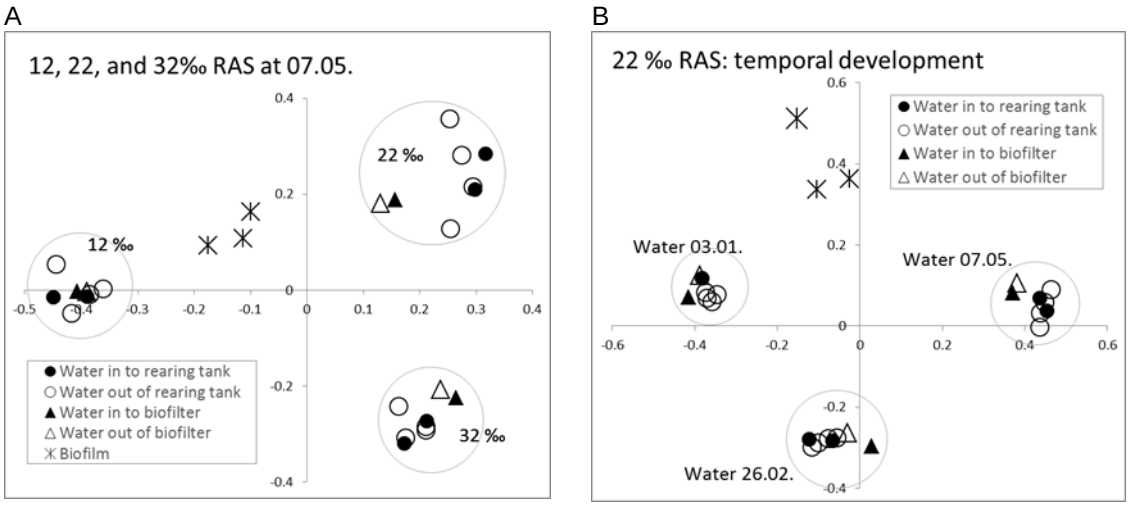

Figure 1. PcoA ordination based on Bray Curtis similarities for microbial community profiles of water and biofilm samples from the 12, 22, and 32‰ RAS at 07.05. (A) and from 22‰ RAS at three dates (B).

Within each RAS, the microbial communities associated with water appeared to be similar throughout the system (Fig. 1A): The microbial communities of the rearing water were similar to those of the bioreactor water. Moreover, the microbial communities of water going in to the bioreactor were similar to those of the water going out. Thus, water communities were apparently little influenced by the bioreactor treatment. However, they appeared to be dynamic with respect to salinity (Fig. 1A) and time (Fig. 1B), as significant differences were found

between water communities representing distinct salinities or sampling times. Biofilm communities generally differed from water communities, and appeared to be less dynamic than the water communities. They were more stable over time and less influenced by salinity (Fig.1).

Acknowledgements: This study was funded by the Research Council of Norway (project 217502/E40 Optimized Postsmolt Production “OPP”), and Marine Harvest Norway, Lerøy SeaFood Group, Smøla Klekkeri og Settefisk, Grieg Seafood, Lingalaks, and Erko Settefisk.

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Microbial influence in different rearing systems: flow through, microbially matured and recirculating aquaculture systems

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ABSTRACT

Fish in intensive aquaculture will continuously be exposed to flows of bacteria of different origin and these flows are dependent on the water flow rates and bacterial biomass of the process water, by bacteria added along with live feed and microalgae, and by internal sources from bacterial growth in the tanks. The treatment and maintenances of the water supply to tanks and the aquaculture system, contribute to differences in the bacterial numbers in the inflowing water and in the water of the tanks. The environmental conditions of the tanks, including the surface area and the supply of organic substrates, may affect the internal processes of bacterial growth. The microbial community of the tanks will not be reduced through the process of water exchange unless the contribution from internal bacterial growth is high as compared to other bacterial flows. The importance of internal growth processes is therefore expected to be highest when the dilution rate of the tanks is low. For very high dilution rates, the importance of internal bacterial growth for the overall flow of bacteria is expected to be low and increasingly dependent on growth of bacteria that are attached to surfaces of the system. The composition of the microbial community in the biofilms of aquaculture systems typically differs from the microbial composition of the water.

A quantitative spreadsheet analysis has been undertaken of bacterial flows and exposure to host larvae in aquaculture systems that are used in larviculture: flow through systems, flow through systems that receives microbially matured water and recirculating aquaculture systems (RAS), with the input of data that were measured in the different systems combined with relevant literature values.

It was notable that the microbial community of the process water in RAS was almost totally dominant for the overall flow of bacteria in tanks of this aquaculture system. Experimental measurements showed that the microbial community of water in RAS rearing tanks was more similar to the microbiota of the incoming water than the microbiota of the live feed. In contrast, the flow through system showed the opposite situation. The situation for RAS offers options of controlling and steering the microbial conditions of the tanks through appropriate manipulations of the process water, which can be an important option for microbial control. The initial situation can also be relatively well controlled in all systems by careful preparation and manipulation of the bacterial community.

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Microbiota in recirculating and semi-closed aquaculture systems for post-smolt production

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ABSTRACT

The microbiota in closed aquaculture systems are important for water quality, but are also a potential threat for robustness and health of the fish. In this study, we have investigated the dynamic changes of the microbiota in a semi-closed aquaculture and in a recirculating aquaculture system (RAS) during post-smolt production of Atlantic salmon. Next-generation sequencing of bacterial 16S rRNA genes was used to analyze the microbiota of water and biofilm samples, i.e. biofilms from the tank walls and the biofilters in the semi-closed system and the RAS, respectively. Overall, the microbiota composition were indeed different between the two aquaculture systems. Also clear differences in compositions between the water and the biofilms within each system were observed, though less different in RAS. Temporal fluctuations of the microbiota composition and diversity were especially observed within the semi-closed system, likely due higher turnover of the water compared to the water in RAS. It could be hypothesized that a RAS is prone to obtain a more stable microbiota with higher interaction between the microbiota in the water and the biofilters. Further studies on the microbiota in these closed aquaculture systems are needed to obtain better knowledge and control of their impact on post-smolt production, robustness and health.

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Microbial water quality dynamics in RAS during system start-up

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ABSTRACT

Microbial water quality in recirculating aquaculture systems (RAS) is although important for successful RAS operation still difficult to assess. There is a need to identify factors affecting the bacterial dynamics (abundance and activity) to ensure microbial stability in RAS. Different culture dependent techniques are used to quantify and identify microbial species, however it is generally time-consuming and often representing less than 1% of the microbial population. Simultaneously with measurements of ammonia, nitrite and nitrate to document the achievement of system steady state, we investigated the bacterial dynamics in the water phase from different RAS by use of a patented method that rapidly quantifies bacterial activity in water.

The study included six identical pilot scale RAS of 1.7 m^3, each stocked with 20 kg rainbow trout (*Oncorhynchus mykiss*). A rapid, culture independent method – Bactiquant® - was used to quantify bacterial activity and estimate the temporal dynamics in the water phase during system maturation. Selected biological and chemical water quality parameters were included to investigate relationships with BactiQuant®. Water quality and fish performance was investigated during a period of two months after stocking fish into the six RAS.

The results showed a more than ten-fold increase in bacterial activity during start-up (average Bactiquant values (BQV) below 3,700 at Day 0 and above 53,000 at Day 24). After three weeks, BQV declined and stabilized. The study revealed considerable variation in initial BQV levels between RAS; over time these differences diminished. Highly significant correlation between BQV and BOD were found. TAN, nitrite and nitrate levels were similar in all six RAS, it was not related to and did not affect bacterial water quality.

Low levels of TAN and nitrite and predictable levels of nitrate were found in all six RAS, however Bactiquant measurements revealed substantial fluctuations in microbial activity over time and between RAS, especially in the initial phases. The study illustrates the bacterial dynamics in RAS during start-up and emphasizes the need for more knowledge of factors affecting microbial water quality in RAS.

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Crossbred fish will lower the risk of disease outbreaks and increase the eyed-egg supply for RAS-facilities

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ABSTRACT

We expect that crossbred fish will out perform purebred fish for resistance to disease, which will result in a lower risk of disease outbreaks in RAS-facilities, in addition to being a product available all year around. The reason is that crossbreeding exploits heterosis, resulting in more robust fish both regarding to survival and disease resistance. This breeding strategy is well-known for improving fitness results when genetically-different populations are crossed to generate F1-generations. The sudden change from well established homozygote genotypes in different allele variants for the two populations, results in a sudden increase of heterozygosity, and higher genetic variation. Commonly the heterosis effect will be most visible in traits where the dominance effect is large. This is typically traits with low heritability, as reproduction and survival related traits.

Robust fish with high adaption abilities are those that are best suited for a RAS-facility. Even though RAS-facilities are known for its clean environment, a challenge is the expenses over a complete production stop if an outbreak of a disease occurs, and the facility needs a total disinfection. In that case, robust fish is needed to prevent outbreaks, and crossbred fish could be a good solution to achieve even more robust fish compared to what is present at the marked at the moment.

In order to get top economically gain doing smolt production in RAS-facilities, the producers are dependent on a year around eyed-egg supply. By producing roe at indoor-facilities for the maternal population and either freeze milk off-season or produce milk in indoor-facilities as well for the paternal population, crossbred eyed-eggs will be a year around product, giving RAS-facilities the possibility to supply new batches of eyed-eggs continually. Therefore, crossbred eyed-eggs will fall naturally as a number one product for RAS-facilities, both due to performance in fitness traits and its possibility of being delivered all year around.

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Practical experiences with smolt system startup and operation

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ABSTRACT

When a newly build fish farm is to be put into operation and fish production starts, it is extremely important that the fish thrive and perform well from the very start. One important issue here is the biofilters and their function and operation. The nitrification process is off course important but certainly not the only area of concern. In the presentation I will discuss different important aspects related to start-up from a practical, hands-on perspective.

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Atlantic salmon post-smolts in RAS: effects of salinity, exercise and timing of seawater transfer on performance, physiology and welfare

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ABSTRACT

Around 16 % of the Atlantic salmon transferred to sea pens is lost in Norwegian salmon production, and most of the losses occur shortly after sea water transfer. Producing a larger and more robust post-smolt in recirculating aquaculture systems (RAS) or semi-closed facilities in the sea may reduce mortality and shorten the production time in the sea and thus reduce the problems with sea lice. However, the biological requirements for optimal performance, health and quality of large Atlantic salmon post-smolts in RAS are not known.

The effects of salinity, water velocity and fish size on seawater tolerance, physiology and performance was studied in two trials. In trial 1, Atlantic salmon smolts (70 g) were stocked in 3.2m^3 tanks in three RAS systems with salinities of 12, 22 and 32‰ and subjected to either high or low water velocity in the tanks (1.0 and 0.3 body lengths per second). When reaching an average weight of 250 and 450 g, post-smolts were transferred from 12 and 22‰ to seawater RAS (32‰). At an average weight of approximately 800 g, all fish were transferred to tanks with flow-through seawater. In trial 2, Atlantic salmon smolts (190 g) were stocked in 3.2m^3 tanks and exposed to 12‰ in RAS. At an average weight of 600 and 1000 g fish were transferred to seawater pens (125 m3) and feed intake, growth, welfare and quality was studied.

Lower salinity had positive effects on survival, growth, feed utilization and pigmentation, and exercise increased growth rate in all salinity groups. Skin histology and a higher expression of stress induced genes in skin indicated a negative effect of higher salinity on skin health. Sexual maturation measured as GSI was low and not affected by salinity or exercise. However, post-smolts at 32 ppt developed cataract earlier and had higher plasma cortisol levels compared to fish at lower salinity. Post-smolts transferred to sea pens at 600 g started to feed within two weeks after transfer while post-smolts transferred at 1000 g had low feed intake for a period of 8 weeks after transfer. Overall survival was >98% in all treatments. The results from this study show that the losses in Norwegian salmon production can be reduced by combining land-based production of large post-smolts with production in traditional sea pens. However, further studies are necessary to develop production protocols to maintain optimal health and quality of large post-smolts in RAS.

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Comparing the effects of high versus low nitrate on post-smolt Atlantic salmon performance and physiology in RAS

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ABSTRACT

Recirculating aquaculture systems (RAS) are designed to control and optimize water quality for the culture of a variety of aquatic species. However, in many RAS, particularly those not equipped with denitrification technologies, nitrate accumulates as an end-product of nitrification and is only controlled by the feed loading rate of the system. Recent research indicates that accumulating nitrate can be harmful to certain fish species produced in RAS, such as rainbow trout. However, very little research has been conducted to evaluate the long-term effect of accumulating nitrate on Atlantic salmon cultured in RAS; hence, an upper threshold for safe culture of Atlantic salmon has yet to be established.

Thus, an 8-month trial is underway at the Freshwater Institute to evaluate the effects of "high" (100 mg/L) versus "low" (10 mg/L) nitrate-nitrogen (NO_3-N) on post-smolt Atlantic salmon cultured in RAS. Atlantic salmon were stocked in equal numbers into 6 replicated RAS (9.5 m^3 total volume). The salmon were 102 ± 1 g when the study began. Sodium nitrate was continuously dosed to three RAS to maintain the target concentration for the high NO_3-N treatment. Low NO_3-N treatment conditions were maintained in three RAS via nitrification, as well as by constant dosing of sodium sulfate to balance conductivity. Each experimental RAS was continuously flushed with 3.7 L/min of water, which resulted in a hydraulic retention time of 1.7 days. The study has been underway for 7 months.

Results to date indicate that the NO_3-N levels under evaluation do not negatively impact Atlantic salmon performance. After 6 months, the mean weight of Atlantic salmon cultured in the high and low NO_3-N RAS was 911 ± 19 and 927 ± 10 g, respectively. Survival to date, excluding culls for sampling and several fish that jumped out of tanks, has been excellent for the high and low NO_3-N treatments, i.e. 99.4 ± 0.5 and 99.2 ± 0.2%, respectively. Cumulative FCR over the first 6 months of the study for the high and low NO_3-N treatments was 0.99 ± 0.02 and 0.98 ± 0.01, respectively. Abnormal swimming behavior has not been observed for Atlantic salmon exposed to high or low NO_3-N.

A full report of the completed 8-month study will be available at the time of the conference. Based on available data, the results indicate that post-smolt Atlantic salmon can be safely cultured in RAS at NO_3-N concentrations up to 100 mg/L under conditions similar to those used during the present study.

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The influence of nitrate nitrogen on post-smolt Atlantic salmon (*Salmo salar*) reproductive physiology in replicated RAS

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ABSTRACT

A major issue affecting land-based, closed containment Atlantic salmon (*Salmo salar*) growout production is precocious male maturation, which can negatively impact, among other things, feed conversion, fillet yield, and product quality. Sexual maturation in Atlantic salmon is a complex process, and its initiation can be influenced by numerous environmental factors; therefore, there is a need to identify and control critical factors that promote salmon maturation in order to reduce or eliminate this problem in water recirculation aquaculture systems (RAS).

Along with other water quality parameters, elevated nitrate nitrogen (NO_3-N) has been shown to influence the reproductive development and endogenous sex steroid production in a number of aquatic animal species, including Atlantic salmon. Without denitrification, low-exchange RAS can accumulate NO_3-N, often to levels >75mg/L, and with this in mind we sought to determine whether elevated NO_3-N in RAS can influence early maturation in post-smolt Atlantic salmon.

An 8-month trial is currently (June, 2015) underway at The Conservation Fund's Freshwater Institute to assess the effects of NO_3-N on a variety of performance and physiological outcomes, including those related to sexual maturation. Post-smolt Atlantic salmon (102 ± 1 g) were stocked into six replicated RAS, with three RAS randomly selected for dosing with high NO_3-N (100 mg/L) and three RAS set for low NO_3-N (10 mg/L). At 2-, 4-, 6-, and 8-months post-stocking, 5 fish were randomly sampled from each RAS, gonadosomatic index (GSI) data were collected, and samples of plasma, brain, hypothalamus, pituitary, liver, and gonad were harvested for measurement of sex steroids and expression of transcripts associated with the onset of puberty. As well, at 4- and 8-months post-stocking, samples of culture tank and spring makeup water were collected and tested for a range of hormonally active compounds using mass spectrophotometry.

At the time of abstract submission, gene expression and waterborne hormonally active compounds data are still forthcoming, and will be presented at the NordicRAS Workshop. Preliminary data indicate that while early male maturation is relatively prevalent (approximately 60-70% of males sampled) in the overall study population, there does not appear to be a significant treatment effect indicating NO_3-N influence on male maturation prevalence (mean (± SE) GSI in high NO_3-N treatment males = 3.86 ± 1.44, vs. 4.83 ± 1.27 in low NO_3-N males; p=0.311), which suggests that other culture parameters are likely instigating early maturation. Final results will be presented in September, 2015.

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Performance improvements with stable pH values in RAS

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ABSTRACT

In recirculating aquaculture systems, pH can vary greatly over short periods of time. pH vary both due to the origin of the inflow water but also the production in the system, where both the metabolic activity of the fish the microbial processes in the biofilters result in changed pH levels over time. The fish will physiologically counteract these variations and even small variations in pH have proven to affect performance.

To quantify the performance losses, due to changing pH levels, tests have been performed under both laboratory conditions at BioMar's test facilities in Hirtshals and on fish farms with extensive recirculation. We make the quantification by adjusting the pH to a predefined level. Further we tested different methods for pH control: single daily spiking of pH or as instantaneous response to pH variations. By securing optimal and stable pH, the stress experienced by the fish is eliminated. This in the end, results in significant improvements in feed utilization, both seen as increasing growth rates and also improving the feed conversion rate. By having optimal pH levels can result in a substantial economical profit for the fish farmer.

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Monitoring water parameters, fish health and welfare in production scale RAS – a follow up study

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ABSTRACT

Aiming to increase knowledge and documentation on fish health and welfare issues in production scale RAS, Finnish Food Safety Authority Evira, Arvo-Tec Ltd. and VTT Technical Research Centre of Finland conducted a research project called "On-line water parameter monitoring and fish health in production scale RAS". The project consisted of two parts: 1) finding suitable sensors for on-line water parameter monitoring (most importantly ammonia, nitrite, nitrate, carbon dioxide and suspended solids), and 2) finding causality between water parameters and possible disease and/or change in welfare indicators in rainbow trout. This presentation covers the results from the second part of the project. The study, funded by the European fisheries fund, was performed in a rainbow trout fingerling and food fish producing production scale recirculation farm. Fish health and welfare parameter changes such as histopathological changes in gills and inner organs, bacteriological isolations, parasitological findings and fin lesions were monitored. These findings were compared with water parameters (oxygen, temperature, pH, ammonia, nitrite, nitrate, carbon dioxide and suspended solids) measured. Results from one pre-grow out production cycle (time period of 7 months) will be presented.

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The automatization of the water quality monitoring in recirculation aquaculture systems (RAS)

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ABSTRACT

Water quality monitoring is an integral part of the daily RAS management. It is both time consuming and often requires skilled personal with knowledge in water chemistry. The measurement of nitrite and ammonia is especially challenging. Majority of available in-line probes, currently on the market are not custom made for use in aquaculture systems and are often unreliable.

A concept for a customized miniaturized automated colorimetric analyzer (MACA; Philips, Netherland) for the measurement of total ammonia nitrogen (TAN) and nitrite-nitrogen (NO_2-N) in aquaculture is developed within the AQUAlity EU FP7 project for SME-associations and was tested together with commercially available multi-sensor platform for continuous monitoring of water quality in RAS in this study.

The performance of the multi-sensor platform was tested in the Nofima Centre for Recirculation in Aquaculture in Sunndalsøra during Atlantic salmon smolt production. The platform consisting of the Pacific control unit (Oxyguard, Farum, Denmark) and five in-line probes (salinity, oxygen (O_2), pH, total gas pressure (TGP), carbon dioxide (CO_2)) was installed in the degassing sump for continuous measurements of the water quality within RAS. The accuracy and the measurements range of MACA were tested mainly in the lab conditions and by the end of the experiment the unit was connected to the platform for continuous measurement of NO_2-N in operating RAS.

The accuracy of the in-line probes was significantly affected by the biofilm formation on the measurement surface indicating the need for daily cleaning routine in the systems with high organic load. Probe cleaning system using compressed air has improved the stability of pH and oxygen measurements over time. The accuracy of the NO_2-N measurements was in the range between 0.05 and 2.5 mg/L of NO_2-N. The continuous use of MACA in RAS provided measurements of NO_2-N in intervals of 19 minutes and the range of measurements was between 0.11 and 0.21 mg/L NO_2-N. The accuracy of the TAN measurements was in the range between 0.1 and 5 mg/L TAN. Further development of MACA for commercial use is currently on the way.

Acknowledgements: The research leading to these results has received funding from the European Union's Seventh Framework Programme managed by REA Research Executive Agency (FP7/2007-2013) under grant agreement number [286995] in a research for the benefits of SME associations, project AQUAlity: "Multi-sensor automated water quality monitoring and control system for continuous use in recirculation aquaculture systems".

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A sustainability evaluation, based on environmental indicators, of Recirculating Aquaculture Systems (RAS) applied to all countries and all species

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ABSTRACT

The aquaculture industry´s continued growth and development hinges on the sustainability (economical, environmental and social) quality assurance of fish farming projects. Recirculating Aquaculture Systems (RAS) are recognized to result in relatively fewer environmental impacts as compared to other methods of aquaculture production. RAS treat effluents prior to their discharge and thus minimize several risks such as the release of active chemicals and the escape of live animals; additionally, water volumes utilized in RAS are much lower in comparison with other production systems. As RAS are considered "closed" systems, there is little to no interaction with surrounding habitats, which results in reduced risk of disease and pathogen transfer to wild populations, as well as lowered incidents of wildlife and predator mortalities. On the other hand, feed and energy use in RAS are considered the main environmental impacts and represent the greatest threats to the environmental sustainability in the short-long term. The following study-review defines and defends several environmental sustainability criteria by which RAS are evaluated for the purpose of developing decision-making recommendations for public bodies, consumers and/or businesses.

A literature review and a technical assessment of the environmental constraints (or impacts) of a generic RAS facility was completed during the following study. The evaluation was based on a realistic, yet precautionary, approach with respect to variations between RAS facilities and the wide variety of species that can be cultured in them. As such, the assessment accurately captures the environmental impacts of a RAS facility culturing any species and operating in any country around the world. RAS were assessed according to several distinct categories of environmental impacts (or risk of impact), including effluents, habitats, chemical use, feed and marine resource utilization, escapes, disease, source of stock, wildlife and predator interactions, and the introduction of non-native organisms (other than the farmed species). Energy use in RAS was also assessed.

Overall, RAS are shown to mitigate many of the environmental impacts associated with other aquaculture production systems (e.g., net pens, ponds, flow-through systems). Energy use remains one of the principal concerns and a conclusion of the presented study is that energy consumption should be the focus of further research and scrutiny. However, in general, RAS reduce or eliminate many of the environmental concerns associated with commercial aquaculture.

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Co-culture of Japanese short-neck clam (*Ruditapes philippinarum*) and sea cucumber (*Apostichopus Japonicus*) by feeding *Pyropia spheroplasts* based diets in Recirculating Aquaculture System (RAS) - a preliminary report

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ABSTRACT

Recently, there has been much emphasis on developing sustainable approaches for aquaculture. The importance of integrated aquaculture for marine organisms has been realized increasingly in the last decade.

Three replicates having similar sized 20 clams (mean shell length 34 mm) and 2 sea cucumbers were distributed 10L fibre glass tank with thermo-controlled filtered sea water (15±1℃) and conditioned for 10 days. Plastic nylon bags were used for keeping the clams. Four different *Pyropia spheroplasts* (PS) based diets named PS-WRP, PS-BRP, PS-RRP and PS-SBP were given at the rate of 3.0 % body weight at 11.00 and 14.00 hours at ad libitum for 42 days.

No negative sign was observed among all the dietary treatment and mortality was low and independent. Higher clam survival was obtained in SBP group followed by BRP, WRP and RRP. A significant ($P<0.05$) higher carcass ratio was obtained in SBP compared to other groups. No remarkable changes were recorded in length, width and depth of the test clams of different test groups. The sea cucumbers fed PS diet containing soybean (SBP) enhanced higher growth compared to WRP, BRP and RRP. It indicated that the sea cucumbers ingested uneaten SBP diet and feces effectively which resulted better weight gain. Therefore, SBP was found suitable over WRP, BRP and RRP diets. No mortality of the sea cucumbers were recorded during the whole experimental period.

The results revealed that clams and sea cucumbers can be co-cultured in CRS without compromising the growth.

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Salmon farming -an integrated research project on land-based aquaculture systems in Norway

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ABSTRACT

The global demand for fish is rising, caused by population growth and an increasing per capacity consumption of fish. Aquaculture farming is an alternative to traditional capture fisheries, giving an opportunity to meet the growing demand without contributing to over-fishing the seas. In Norway, aquaculture was introduced in 1970. Since then fish production has become an important factor for the Norwegian economy. The industry does however have a large impact on the environment, for instance through pollution, spread of disease and fish escaping from the production sites. Future developments in the fish farming industry need to ensure a more environmentally-friendly production. Technical advancements have introduced the opportunity to move production from sea to land. A carefully managed land-based farm could give the opportunity to produce large quantities of biomass in a controlled environment. Land-based aquaculture is however capital intense, requiring large initial investments as well as high operational costs. Additionally, the biological production process is related to high risk. The remaining question is if these facilities can be constructed in a way that makes them economically sustainable.

This research project focuses on land-based aquaculture using RAS technology. A system dynamics approach is used, and a quantified simulation model of a land-based aquaculture system built. The model integrates separate parts of a land-based facility, exploring the behaviour arising when the different components interact over time. This gives an opportunity to conduct an integrated analysis of the economic feasibility of these systems.

The results suggest that a land-based facility can be economically sustainable, in the sense that it can generate a positive EBIT and cover an initial loan solely by the revenues generated by the firm. The loan coverage is 97 million NOK in the base-run scenario. In case of a favourable price development of salmon, or if the firm manages to lower the cost of important production inputs, the loan coverage can increase up towards 187 million NOK. The worst case scenario generates a negative EBIT and loan coverage of zero. The results do also show that the production cost per kilogram biomass produced is lower than the production cost in a sea-based facility in the base run scenario. The financial analysis does not include the monetary aspect of the environmental impact of production. If it did, the perceived feasibility of land-based production could potentially be higher, both from the perspective of an individual firm and for society at large.

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Ponds, Raceways, RAS - benchmarking trout grow-out economics

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ABSTRACT

Ponds, raceways, net cages, recirculating systems; the variety of trout production methods is truly impressive. While conventional flow-through systems pre-dominate still the European trout sector, there seems to be an ongoing trend of the adaption of recirculating aquaculture systems (RAS) in northern countries. Proponents of RAS highlight its efficient production method due automation and its resource-friendly recirculating practice. From their perspective, RAS is seen as the future of trout farms. In contrast, sceptics criticize the high investment and energy costs of those high-tech farms, which would endanger the long-term profitability of the RAS technique. But, for the analyses of the competitiveness of different production systems, reliable and international comparable data, at best, economic performance data at farm level is needed. Unfortunately, detailed economic data of trout farms are often not at hand in every country.

Using the `typical farm approach' we promote a more qualitative approach for benchmarking different kinds of trout farms. Based on focus groups with local trout farmers, consultants and researchers and expert interviews as well as field observation we define farm models and ground our farm datasets empirically in Denmark, Turkey and Germany. Thus, our sample includes two export-orientated trout production nations and one important market for portion-sized trout. Following the qualitative procedure datasets about in- and outputs of typical farms are provided. One of the advantages of this approach is the reduction of costs for the collecting data, while the in-depth data analyses simultaneously lead to an enhanced understanding of farm economics.

Our farm level benchmark focuses on cost-efficiency at the grow-out stage to make the production systems analyses international comparable. Turkish farms which use simple concrete ponds have the advantage of low investment costs and wages, high withdrawal rates and exchange rate effects. Danish RAS farms are highly productive. German farms which use raceways are productive as well and are able to take advantage of high prices at local markets. Our presentation will enable a detailed look at the separate cost structures and thereof the resulting long-term profitability of each farm type. Our presentation will further explain and discuss the potentials and limitation of using a data collection system based on 'the typical farm approach' for future data collection.

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Woodchip denitrification bioreactor nitrate and solids removal from RAS wastewater

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ABSTRACT

Woodchip bioreactors have proven to be a simple and relatively inexpensive option to remediate nitrate in agricultural waters and effluents, and this technology now holds potential to treat outflows from recirculating aquaculture systems (RAS). However, RAS wastewater generally has higher chemical oxygen demand (COD) and total suspended solids (TSS) than previous bioreactor applications such as the treatment of subsurface drainage water. These wastewater constituents may lead to clogging in these plug-flow style designs due to the physical filtering ability of the woodchips.

Four 1:10 pilot-scale woodchip bioreactors (L x W x D: 3.8 x 0.76 x 0.76m) were constructed and operated at The Conservation Fund Freshwater Institute, Shepherdstown, WV, USA to assess (1) water treatment beyond nitrate removal (i.e., COD and TSS removal) and (2) the potential for woodchip clogging to impact bioreactor hydraulics.

The bioreactors were operated under a range of hydraulic retention times (5.8 to 62 h) with 90% nitrate-N removal occurring roughly on the order of 36 to 48 h of retention. The woodchips released an initial COD flush, but the bioreactors transitioned to COD removal by the time the 90th cumulative pore volume had passed through (which ranged from day 40 to day 113 depending on the flow rate). The bioreactors provided greater than 90% TSS removal which primarily occurred near the inlet manifold.

Over time, there was evidence of modified hydraulics for water being pumped into the bioreactors as the rate of inflow into the chips slowed. Greater hydraulic loading led to more cumulative removal of COD and TSS, but also more altered hydraulics.

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End-of-pipe removal of nitrogen using woodchip beds

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ABSTRACT

Nitrogen (N) removal from aquaculture effluents is currently facilitated mainly by denitrification reactors. These reactors require constant input of readily available carbon (C) sources to fuel the denitrification process, which can restrict their application in aquaculture systems due to practical and/or economic reasons.

An alternative technology for removing N, which has been used for more than two decades to treat surface and drainage water, is based on using wood by-products as solid C sources for denitrification. In these denitrifying beds, wood-based substrates are implemented into the ground or filled into containers to intercept the waste stream or treat point source discharges, respectively. The technologically simple systems are characterized by low-maintenance and high longevity rendering them potentially relevant for end-of-pipe treatment in aquaculture.

Based on pre-assessments in the laboratory, the current study set out to investigate the interactive effects of two operating variables; hydraulic retention time and bicarbonate addition (inlet concentration), on N-removal rates. Experimental, horizontal-flow woodchip filters treating the effluent from a freshwater recirculating aquaculture system were used in the study.

Results demonstrate that the woodchip beds were able to remove nitrate-N sustaining that denitrification occurred within the woodchip filters. In addition, nitrate removal could be enhanced by addition of bicarbonate. The study thus indicates that nitrogen removal through denitrifying woodchip filters may present an alternative end-of-pipe treatment method for N-removal in some aquaculture facilities.

Acknowledgements: This research was funded by The Ministry of Food, Agriculture and Fisheries of Denmark and by the European Union through The European Fisheries Fund (EFF).

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Comparison of Atlantic salmon postsmolt (*Salmo salar*) produced in recirculating aquaculture systems (RAS) and a traditional sea cage

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ABSTRACT

Lerøy Midt AS is looking into the possibility of producing postsmolt in landbased facilities using recirculating aquaculture technology (RAS). Transfer of larger smolts reduces the production time in sea, and could have several positive effects such as less exposure to salmon lice, improved biosecurity, more robust fish and reduced mortality, faster growth rate, and improved fish welfare. Production of post-smolts in RAS is under evaluation, and the potential challenges associated with this type of production, such as early sexual maturation and maintenance of proper water quality, need to be evaluated. The aim of the project was to fill some of the knowledge gaps associated with postsmolt production in RAS by investigating fish welfare and health, technological and operational challenges, and economic considerations.

This study was performed at Lerøy Midt AS, location Lensvik, starting 7/4 2015 and ending 14/5 2015. Atlantic salmon smolts were stocked in 6 tanks (100 m^3), with brackish water (12-14‰), continuous lightning and 12 C°. Fish average weight at the beginning was 134±23g, and the density was 36 kg/m^3. Fish density before transferring to sea was below 66 kg/m^3.

The main and makeup water flow were set to 960m^3/h and the 27 m^3/h, respectively, resulting in a water exchange rate of 84% of total system volume, and a tank water retention time of 35-40 minutes.

Water quality parameters (O_2, CO_2, TAN, salinity, pH, alkalinity, temperature, TSS, turbidity, organic material), fish performance (growth, survival) welfare indicators, gonado-somatic index and water velocity in the tanks were measured throughout the experiment. A comparative group of salmon smolts produced at Lensvik was transferred to sea and was used as a control group. This group was monitored with respect to lice, survival, and growth, and a comparison with the on-land produced post-smolts in RAS will be done up to the slaughter size.

The results from the different water quality measurements, fish performance and tank water velocity during production of post-smolts in RAS will be presentation at the conference, as well as the data from the comparison with the control group transferred to the sea.

Acknowledgements: This study was funded by VRI Trøndelag and Lerøy Midt.

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Scaling of culture tanks and unit processes, relevant for Atlantic salmon post-smolt production in land-based systems

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ABSTRACT

Norwegian authorities have envisioned an increase in salmon production to 5 million tons by the year 2050. However, challenges related to sea lice, escapes, and fish mortality during the sea phase may hinder this development. Recent regulatory changes to combat these issues, such as increases in allowable fish size in land-based facilities, and land-based licenses without fees, have generated considerable interest in development of these systems for Atlantic salmon.

Such a change in production strategy must to be supported by research on post-smolt performance, welfare and health, as well as on water and biofilm microbiota, and design and scaling of water treatment unit processes and culture tanks. In this talk, findings from several projects relevant for land-based post-smolt production will be presented, as well as plans for future research.

Several experiments were done on effects of scale or intensity in land-based systems, including: 1) effects of tank volume (scaling) on salmon post-smolts, 2) effects of moving bed bioreactor (MBBR) scaling, air flow and TAN, and 3) effects of low and high post-smolt density in RAS and flow-through systems. Response variables were water quality, TAN removal efficiencies, growth, survival, and various physiological indices. These experiments were part of the EU project AquaExcel (experiment 1 and 2), in collaboration with partner IMARES (lead of experiment 2), and the Research Council of Norway project SalmoFutura (experiment 3).

Rearing tank scale significantly affected salmon post-smolt growth rate, behavior, and physiology. Post-smolts reared in tank volumes typical of research (1 m^3) showed significantly lower growth rate compared to semi-commercial tank volumes (100 m^3). How even larger tanks affect water quality, hydrodynamics, and fish performance and welfare is unknown, and will be focused on in the newly started CtrlAQUA SFI centre, given that farms for post-smolts are being planned with tank volumes of 1000 m^3.

In the study on effects of MBBR size, it was found that scale had a significant effect on TAN removal rate. In general, the larger the scale the better the performance. TAN removal at small research-scale (0.8 L) was approximately 80% compared to that at medium scale (200 L). The differences between small scale and semi-commercial scale (>20,000 L) were even higher. Possible reasons for these findings, and their implications, will be discussed. Preliminary results from experiment 3, effects of post-smolt density and system type will be presented at the meeting.

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Poster abstracts

Presented at the

3rd NordicRAS Workshop on

Recirculating Aquaculture Systems

Molde, Norway

30 September - 1 October 2015

Carbon dioxide as limiting factor in partial reuse RAS

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ABSTRACT

About two-thirds of the Norwegian production of smolt takes place in intensified flow-through systems applied tank-internal removal of accumulated carbon dioxide (CO_2). At present, such systems represent more than 200 million smolt produced per year. Elevated CO_2 concentrations in the tanks at high water temperature and peak biomass before smolt delivery are a potential risk for the fish's health and welfare.

In general, the ongoing intensification of the systems ('partial-RAS'), characterized by high fish density and low specific water flow, makes high demand of the reliability and efficiency of the employed technology for CO_2 removal. Available CO_2 figures from commercial farms are sparse, but measured peak concentrations above 30 – 40 mg/L in smolt tanks have been reported. As a general guideline, the CO_2 concentration in freshwater tanks stocked salmon and trout should not exceed 15 – 20 mg/L throughout the production cycle.

Newly developed systems demonstrate improved CO_2 removal compared to commonly applied systems. A removal rate of 60 – 70% is currently measured in a pilot aerator at an air:water ratio of 10:1 even at rather low CO_2 levels (ca. 10 mg/L). Moreover, the system is combining CO_2 removal and back-flushing of the aerator's media for fouling control. Fouling problems and reduced efficiency have been a recurring problem in commonly applied aerators.

A recent study indicates that tanks stocked juvenile rainbow trout at temperature above 10 °C should be equipped up-to-date aeration systems for reliable CO_2 control at flow rates below 0.2 – 0.3 L/kg/min.

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A new experimental fresh water RAS-system with emphasis on online water quality monitoring

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ABSTRACT

Following the pattern seen in several countries, more RAS farms are being built in Finland. The largest project aims at 3 milj kg production of rainbow trout, with construction work already ongoing at the Åland Islands. Other 1+ milj kg projects are also being planned in Finland. To provide useful research data for RAS farming, an experimental set-up with ten individual fresh water RAS systems are currently being built at Natural Resources Institute Finland, at Laukaa fish farm.

Each of the 10 systems consists of a fish tank, drum filter, biolfiltration system with four separate chambers, aeration tower, oxygenation system, and pH-control unit. In addition, a radial flow separator with a bypass option is included. All tank effluent is filtrated through Hydrotech drum filters with standard 80 um mesh. Backwash water is discarded from the system without further treatments during regular trials. Biofiltration chambers have both moving bed media and fixed bed media with a total capacity of 2.5 kg feed per day. The four biofilter chambers, each with 120 liter of biomedia volume, can be used simultaneously or individually. In the aeration tower, water is trickled through bio blocks against air current (5:1-10:1 water:air). Fish feeding is controlled by Arvo-Tec automatic feeding system with the possibility to automatically adjust feeding on the basis of water quality measurements. At this stage, no UV, ozone, or end-of-pipe treatment systems are installed.

Each system will have Oxyguard O_2-probe and Franatech CO_2-probe. Experiments are underway to assess sensitivity of S:can optical probe for measurements such as nitrite, nitrate and turbidity at levels typical for RAS-farming. Both recirculating water and replacement water volumes are measured automatically. All measurements, water level indicator and power blackouts are connected to the alarm system with GSM option. Emergency oxygenation starts automatically during power failures. Feeding is controlled by Arvo-Tec feeding system, and the system allows remote control of the feeding.

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The IFREMER Palavas research platform for marine temperate and tropical aquaculture

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ABSTRACT

The IFREMER Palavas research platform was recognized as a large EU research infrastructure in 2001, and since, was linked to the highest class European aquaculture research facilities in the EU projects ASEFAF, AQUAEXCEL and AQUAEXCEL2020.

Part of this infrastructure is currently rebuilt and upgraded, thanks to EU/regional funds through the MeDITERA project (2.3 million €). The objective of this project is to intensify cooperation actions between the production, research and teaching/training sectors around a unique and efficient research "tool" available to regional research and training stakeholders and to Mediterranean and EU aquaculture research communities.

The indoor research facilities upgraded (4000 m^2) in MeDITERA, allow to lead research in the domain of: (1) fish reproduction and genetics (broodstock maintenance, cryopreservation, egg incubation, larval rearing, selected and isogenic lines production and maintenance), (2) fish on-growing (limiting factors, effect of toxicants and alternative feed assessment, RAS optimization) and (3) laboratory facilities (water quality and physiological parameters). New outdoor facilities were also created to develop research on algae production, bioremediation and IMTA systems (2000 m^2).

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